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Institutionen för Cell- och Molekylärbiologi

Regulation of Cellular Responses to Hypoxia by HIF-1alpha-dependent and -independent Signaling Pathways

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Institutionen för Cell- och Molekylärbiologis (CMB) auditorium, Berzelius väg 21

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ABSTRACT

Hypoxia is a state of inadequate oxygen supply to the cells and tissues of the body. It plays a critical role in embryonic development, as well as in various physiological and pathological processes. Hypoxia-inducible factor 1 (HIF-1), a heterodimeric protein complex composed of an oxygen-regulated α subunit and constitutively expressed β subunit (ARNT), is the key regulator of the hypoxia response and regulates genes involved in diverse processes such as angiogenesis, erythropoiesis, glycolysis, pH regulation, apoptosis and cell proliferation/survival. In addition to this “canonical” response, hypoxia can also elicit multiple HIF-1-independent cellular response pathways such as unfolded protein response (UPR). Furthermore, hypoxia can also crosstalk with Notch signaling and augment the Notch downstream response.

The aim of this study was to investigate the molecular mechanisms of HIF-1-dependent and -independent regulation of cellular responses to hypoxia. We aimed to investigate the molecular mechanisms of cross-coupling between hypoxia and Notch signaling pathways, as well as the role of hypoxia in regulation of β -cell death and function.

In paper I, we identified Notch as a novel substrate of factor inhibiting HIF-1 (FIH-1) and characterized the role of FIH-1 on Notch activity as well as the crosstalk between hypoxia and Notch signaling pathways. We show that FIH-1 hydroxylates Notch intracellular domain (ICD) at two residues (N¹⁹⁴⁵ and N²⁰¹²) that are critical for the function of Notch ICD. FIH-1 negatively regulates Notch activity and accelerates myogenic differentiation. Notch ICD enhances recruitment of HIF-1 α to its target promoters and derepresses HIF-1 α function. Notch ICD has a higher affinity than HIF-1 α for FIH-1 and may enhance HIF-1 α function by sequestering FIH-1 away from HIF-1 α C-terminal transactivation domain (CTAD). In paper II, we identified and characterized a Notch-independent mechanism regulating the responsiveness of the *Hairy/enhancer-of-split 1* (*Hes1*) gene to hypoxia. We show that induction of Notch target genes in response to hypoxia is cell type-specific. In P19 cells, hypoxia-induced upregulation of *Hes1* gene expression is dependent on HIF-1, but independent of Notch. Two N-box motifs in the proximal region of the *Hes1* promoter are critical for hypoxia-inducible transcriptional regulation of *Hes1* promoter activity. In paper III, we investigated the molecular mechanism of β -cell death triggered by hypoxia. We show that apoptosis induced by exposure to 1% O₂ in Min6 cells is dependent on UPR activation and C/EBP homologous protein (CHOP) induction, but independent of HIF-1 α . Exposure to 1% O₂ changes levels of expression of several B-cell CLL/lymphoma 2 (Bcl-2) family proteins and activates the intrinsic mitochondrial apoptosis pathway in Min6 cells. Culturing of isolated pancreatic islets at normoxia leads to intracellular hypoxia, CHOP induction, and cell death. We also show that islets of diabetic db/db mouse are hypoxic. In paper IV, we characterized the regulation of Forkhead box O 1 (FoxO1) by hypoxia in β -cells and explored the potential role of FoxO1 in β -cell function at hypoxia. We show that in Min6 cells, hypoxia triggers FoxO1 nuclear translocation. Hypoxia induces FoxO1 protein levels and inhibits AKT-dependent phosphorylation of FoxO1 residue Ser²⁵³. Hypoxia-mediated nuclear translocation of FoxO1 is dependent on mammalian Ste20-like kinase 1 (MST1) kinase, but independent of c-Jun N-terminal kinase (JNK). FoxO1 does not contribute to the reduced cell viability observed at hypoxia.

The results from these studies contribute to a better understanding of HIF-1-dependent and -independent regulation of cellular responses to hypoxia, in terms of cell differentiation, cancer, as well as β -cell survival and function.